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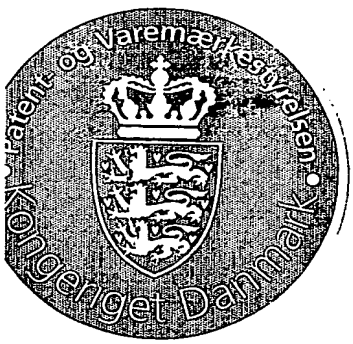

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PATENT- OG VAREMÆRKESTYRELSEN

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The Usage Of Specific Ingredients in Man

by

Kurt Berg

ABSTRACT.

Treatment or upper airways infections (common colds and the like) have turned out to be mostly negative. Therefore, there is a great need for potentiating the various approaches, per se. Very often, the treatments consist of various "active" ingredients such as plant extracts or zinc complexes in lozenges or the like the use of which is thoughts to speed up the recovery.

It is generally believed that the active ingredients cause the faster recovery and that the other pharmacxeutical additives , such as for example sorbitol, different flagrances and or palmitines (for lozenges) play no significant role,although no reports so far has supported this notion for the viruses causing the syndrome known as the "common cold" (for example: rhinoviruses, coronavirus, adenovirus, coxacxkie virus, etc.) .

Recent experiments in our laboratory, however, have shown that these pharmaceutical additives may have important influence on the outcome of common cold treatments.

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Preliminary investigations have demonstrated a surprising effect amongst two of the most used flaglagrence additives used within the field of "common cold remedies", namely Japanese peppermint oil and Menthol as the former upregulate the production of for example rhinoviruses while depressing the interferon alpha system. In contrast, menthol was shown to posses a distinct antiviral activity,per se, and able to potentiate the interferon-α system 2-3 fold at low levels (< 3 unit/ml). This, *in vitro,* was also found in vivo in a limited number of patients as common cold patients receiving otherwise identical treatment with the exeption of mentho vs. Japanese peppermint oil responded differently: those receiving the menthol treatment cleared the common cold faster compared to the group treated with Japanese pepper mint oil.

Example 1. (Figures 1a and 1b)

The antiviral activity of menthol(-) vs. rhinovirus 39 and the potentiation of natural human leukocyte interferon by menthol(-)

Experiment: 1 g pure Menthol(-) from Sigma was dissolved into 3 ml 100% Ethanol and kept at 4° C (Menthol stock solution). Japanese peppermint oil was produced each time from the bottle(stored at 4° C from the supplyer (local hospital pharmacy(RH)). The two stosk solutions were used at dilutions(final) as indicated in the text/figures.

1:800 dilutions of Menthol(-) were added to interferon dilutions on confluent monolayer cultures yielding the interferon units/ml as indicated in Figure 1a and 1b and rhinovirus, RHV-T-39 was added at 10^{-2} dilution(Figure 1 a) or $10^{-2.5}$ (Figure 1 b) diluted from from a virus stock preparation of RHV-39 and the cells were incubated at 33°C for 3-4 days until control cultures infected with the virus alone yielded 100% destruction as seen in a microscope; at that time MTS/PMS was added and the dehydrogenase in the intact cells produced a color which subsequently was

measured in an ELISA scanner as previously described[Hansen, 1989 #85][Berg, 1990 #90]. The results were graphed with the concentration of interferon on the horizontal scale and the OD_{492} readings on the vertical scale.

Results: The lower curve (RHV39 in Figure 1a) illustrates the variation of the virus infection in the individual wells. As can be seen the OD-level is around 0.15 corresponding to a 50-fold reduction in the signal compared to non-infected control cell (black triangle: Cell control(-IFN)); upper curve).

Conclusion: The presence of Menthol, *per se*, yields a significant protection amounting to 10-15% compared to uninfected control cells(cf. protection levels at IFN= 0 units/ml);
At the interferon range between 0 and 8 units/ml the interferon curve+menthol is significantly above the interferon curve, demonstrating a specific potentiation.

Similar results are also seen at a lower virus concentration ($10^{-2,5}$ - Figure 1 b).

Example 2 (Figure 2)

Japanese Peppermint oil increases the growth of rhinovirus 39 and downregulates the protective action of natural human leukocyte interferon

To be forwarded.

Virus titrations

Rhinovirus 1A, rhinovirus 15 and rhinovirus 39 were titrated according to the tetra zolium salt (MTS)-method (Berg et al., 1989; Berg and Owen, 2001a, Hansen et al., 1989). WISH cells were seeded in a micro tray at 3000 cells per well and incubated at 37° C, 5% CO_2 overnight; the following morning the medium was replaced with 10-fold dilutions of either rhinovirus 1A, rhinovirus 15 or rhinovirus 39, respectively, in fresh medium and the trays were incubated 4-5 days

at 33°C; a microscopical examination confirmed that the CytoPathogenic Effect (CPE) was fully developed (CPE equal to 100%). The minimal amount of virus (i.e.: the highest dilution of the virus in question) which produced 100% destruction was used as "challenge virus" in the subsequent experiments. To quantitate the CPE in terms of % destruction, MTS (Berg and Owen, 2001a) was added to all cultures and after 3 h incubation at 37° C (without CO_2) the trays were read in a scanner as previuosly described (Berg et al, 1989, Hansen et al., 1989). Control cell cultures, that were not infected with virus, were included in the experiment; the latter gave the highest OD as these cells were not damaged; depending on the concentration of virus added to the different wells, the OD_{492} varied, accordingly: 100% CPE yielded a low OD(<0.200) ; 0% CPE corresponding to no infection at all (controls cell) gave a high OD (>1.200).

Example 2

No Antiviral activity of ZnGluconate as measured via the MTS-system.

WISH cells were seeded in wells in a microtray and incubated for 24 h at 34 °C, 5% CO_2; the medium was replaced with fresh medium comprising 2-fold dilutions of ZnGluconate (diluted 1:10 from a 1% stock dilutions) and incubated further for 3-4 days at 33 °C, 5% CO_2; on the following day challenge virus was added and after 3-5 days at 33° C, 5% CO_2, MTS was added and the microtray was measured in an OD-scanner (Berg et al., 1989; Hansen et al., 1989). Alternatively, instead of ZnGluconate WISH cells were incubated with other zinc salts/complexes or with the flavonoid derivatives, Troxerutin, Veneruton® or Quercetin.

No substantial protection against rhinovirus by addition of ZnGluconate could be detected (<2% protection). The OD signals from the wells, that were incubated in the presence of ZnGluconate were very close to the virus control curves (Figure 7). Similar results were observed when testing the antiviral effect of other zinc salts/complexes. When the flavonoid derivatives, Troxerutin and Veneruton were added to the WISH cells, they also did not show any antiviral activity (<1% protection). However Quercetin, had a moderate antiviral activity at levels not toxic to the cells (10 to 15% protection).

Example 3

Antiviral activity of Interferon-α (rHuIFN-α-2b) against Rhino virus (1A, 15 or 39).

3.000 WISH cells were seeded in a microtray and on the following morning, the medium was replaced with 2-fold dilutions (from a 0-30 units/ml stock solution) of HuIFN-α-2b (Intron A) in fresh medium comprising 2% serum. After incubation overnight, the medium was replaced with fresh medium comprising challenge virus and incubated at 33° C, 5% CO_2 for 3-5 days and processed further as described in Example 2.

The results in figure 7 clearly demonstrates that rhinoviruses are reasonable sensitive to HuIFN-α-2b (50% protection). However, 90-100% protection can be achieved at approx. 8-15 units/ml.

Example 3 treatment of common cold patients

Patients experiencing the usual common cold symptoms(sore throat, coughing, sneezing, running nose, etc.) for 1-3 days (considered to have acquired the infection within 1-3 days) were treated at the Doctors Office with Venoruton or the equivalents plus ZnCluconate (50 mg added to the usual treatment) using 2-6 mg Menthol per lozenges: 7 out of 7 patient responded to the treatment with a 40-90% reduction in symptom score; this was not seen in a minor study replacing Menthol with japanese peppermint oil.

CLAIMS

Claim 1 The composition of any mixture or any other drug-like entity vs. the common cold syndrome type combined specifically with menthol used as an antiviral entity as far as common colds are related to the usual "common cold viruses" such as rhinoviruses, coronavirus, adenovirus, coxacxkie virus, etc.

Claim 2 The usage of any mixture as mentioned in the preceding claim in which any drug mixture comprises any flavonoid derivative and or any zinc complex

Claim 4 The usage of any mixture as mention in the preceding claims for combating syndromes in which interferon is known to take part in , for example, as a spray, in an interferon lozengers, gel, etc. or other known slow release systems.

Claim 5. The usage of any mixture as mention in preceding claims against allergic/asthmatic syndromes in which interferons are employed as treatment.

Claim 7. The use of any zinc complex as described in the preceding claims in combination with any flavonoid derivative or other derivatives such as rutosides or any analoques (having the same treatment properties) as a treatment in man against The Common Cold Syndrom , per se.

Claim 8. The use of any of the preceding claims in combination with interferon-lozengers or sprays stabilized/potentiated according to the previous claims.

The antiviral activity of natural HuIFN-α vs. Rhinovirus-T39(10^{-2}) incl. M (1:800)

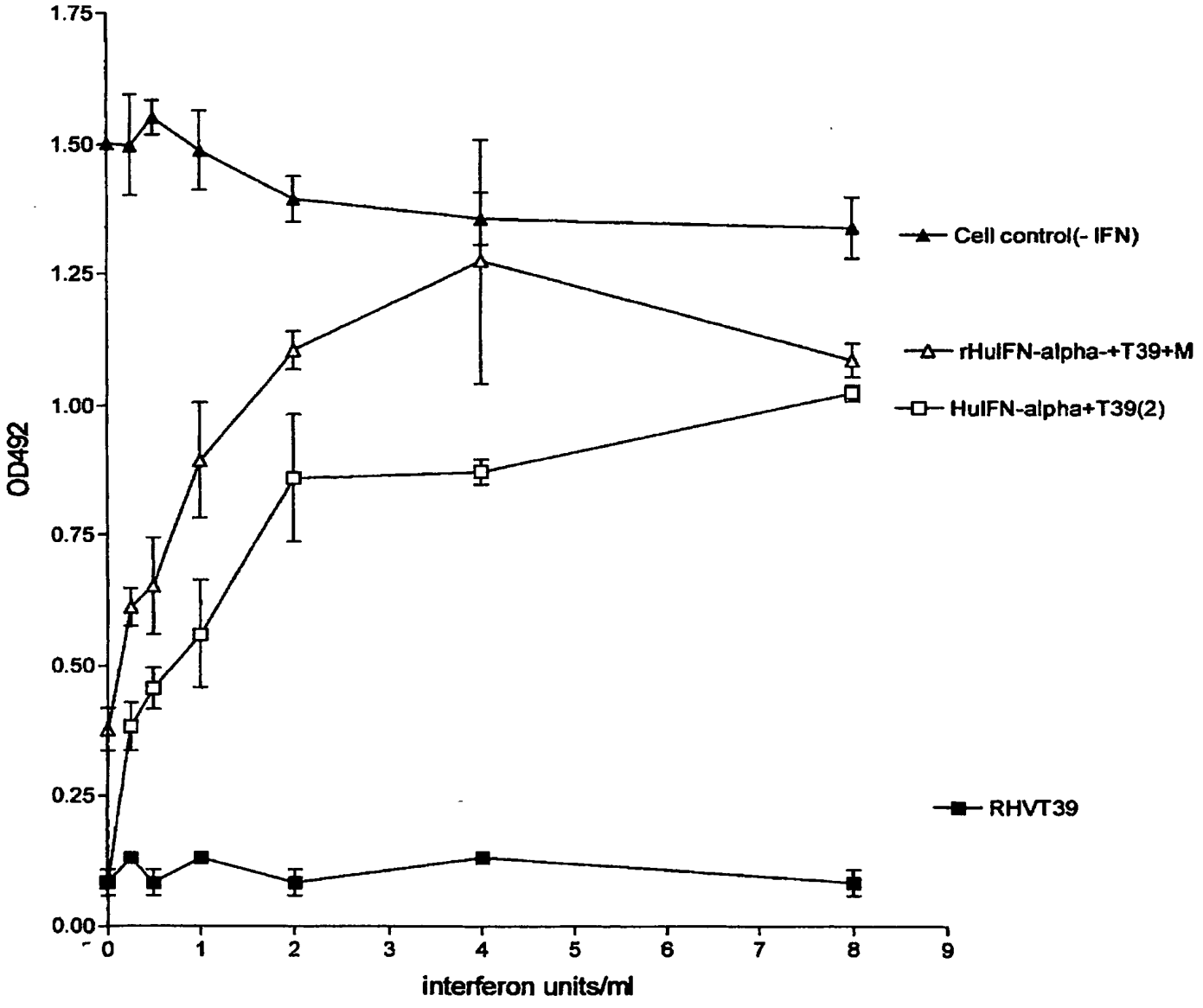

FIG. 1a

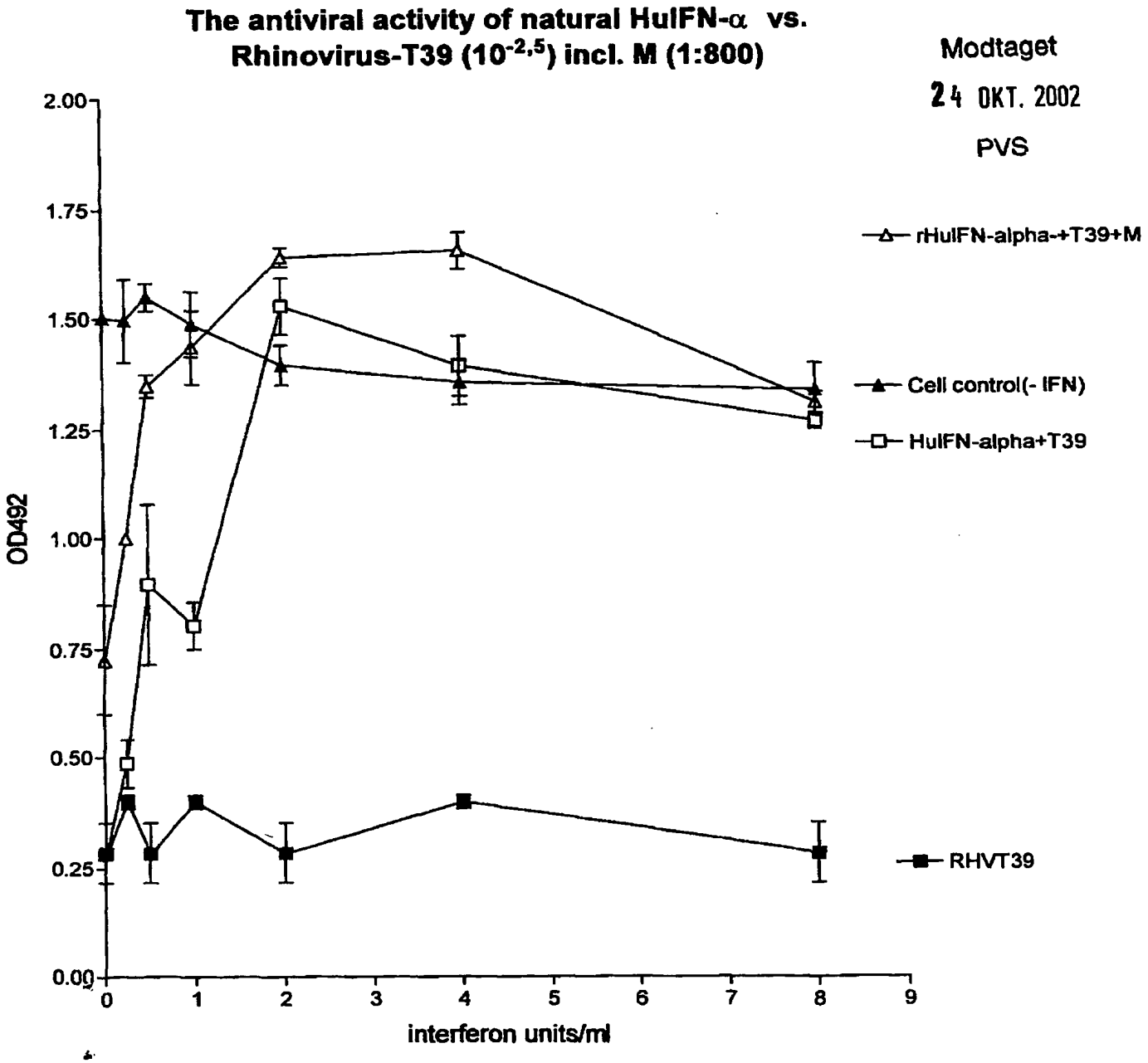

FIG 16